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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

John J. Coogan, Jr., et al. Examiner: Weber, Jon P.

Serial No.: 09/805,610 Group Art Unit: 1651

Filed: March 13, 2001

For: **MONOCHROMATIC FLUID TREATMENT SYSTEMS**

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF BARRY RESSLER PURSUANT TO 37 CFR §1.132

Barry Ressler declares as follows:

1. That I am a joint inventor of the above-identified patent application that was filed in the U.S. Patent and Trademark Office ("PTO") on March 13, 2001, directed to "Monochromatic Fluid Treatment Systems," such application being identified as Serial No. 09/805,610 (the "Application").

2. That concurrently herewith an "Amendment and Response to Office Action Pursuant to 37 CFR §1.111" (the "Response") and a "Petition for Extension of Time" are being filed with the PTO.

3. That this Declaration is provided in support of the Response and is to be considered in conjunction therewith.

4. That as a joint inventor of the Application, I am thoroughly familiar with the invention giving rise to the Application, the content of the Application, and the research efforts associated with the subject matter disclosed and claimed in the Application.

5. That claims 1-9 and 16-20 of the Application are currently rejected by the PTO based on the teachings of U.S. Patent No. 5,730,934 to Holbert (the "Holbert '934 patent"), U.S. Patent No. 5,843,374 to Sizer et al. (the "Sizer '374 patent"), or U.S. Patent No. 6,194,821 to Nakamura (the "Nakamura '821 patent").

6. That with reference to claim 1 of the Application, none of the cited patents teaches or suggests a system for treating a complex fluid that includes, *inter alia*, (i) a non-laser light source for generating and transmitting substantially monochromatic light, (ii) a light emitting surface positioned relative to the non-laser light source for transmission of monochromatic light therethrough , (iii) a complex fluid positioned proximate to said light emitting surface, wherein the complex fluid includes at least one component that is sensitive to a change in temperature; and (iv) a cooling fluid in thermal communication with the light emitting surface, the cooling fluid being effective to prevent the complex fluid from undergoing a temperature change damaging to the sensitive component.

7. That a system for treating complex fluids was fabricated under the supervision of applicants and in accordance with the subject matter of claim 1 of the Application (the "Treatment System"). The Treatment System substantially corresponded to the treatment apparatus schematically depicted in Figs. 1-4 of the Application.

8. That the Treatment System has been used in treating blood components in accordance with claims 1-9 and 16-20 of the Application. Reports related to such testing are appended hereto as Exhibit A and Exhibit B, as follows:

- Exhibit A: Report entitled "T3I – Evaluation of Blood Components Irradiated by a Novel Monochromatic Light Source" prepared by Edward L. Snyder, M.D. et al. at the Yale University School of Medicine, Department of Laboratory Medicine, Blood Bank Research and Development Laboratory (the "Yale Report") (dates redacted);

- Exhibit B: Report entitled "Evaluation of T3I Pathogen Inactivation System Using Porcine parvovirus" prepared by MicroBioTest, Inc., a company

specializing in microbiology and virology laboratory procedures (the "MBT Report") (dates redacted).

9. That with reference to the Yale Report, "the purpose of [the] study was to determine the effects, if any, on blood components irradiated by a novel monochromatic light source," i.e., the Treatment System.

10. That the test procedure giving rise to the results reported in the Yale Report are set forth at pages 2-6 of the Yale Report for fresh frozen plasma, platelet concentrates, red blood cells, Adsol® solution (Baxter International, Deerfield, IL), and packed red blood cells ("PRBCs"). The assay measurement procedures are specifically set forth at pages 5-6 of the Yale Report.

11. That based on the testing reported in the Yale Study, "[n]o clinically significant effect due to irradiation [was] seen on the aliquots of fresh frozen plasma irradiated [with the claimed Treatment System] and stored for 24 hours as performed under the experimental conditions used." [See Yale Report, page 6.]

12. That based on the testing reported in the Yale Study, "[t]here is no clinically significant effect on platelets treated with high, low or medium doses of radiation [supplied by the claimed Treatment System] and then stored for up to 24 hours." [See Yale Report, page 7.]

13. That based on the testing reported in the Yale Study, "[f]or whole blood, radiation at different intensities produced no evidence of radiation-induced damage [with the claimed Treatment System]. Rather, the damage that was seen was attributable to sampling error and time of storage." [See Yale Report, page 8.]

14. That based on the testing reported in the Yale Study, "[f]or Adsol, radiation at different intensities produced no evidence of radiation-induced damage [with the claimed Treatment System]." [See Yale Report, page 9.]

15. That based on the testing reported in the Yale Study, "[p]acked red cells are not affected by T3I radiation using the protocols performed under this trial [with the claimed Treatment System]." [See Yale Report, page 9.]

16. That the authors of the Yale Report summarized their conclusions as follows:

> While admitting that the sample population is small, under the conditions evaluated there appears to be no clinically significant damage inflicted on blood products due to T3I-induced irradiation [using the claimed Treatment System]...Thus, we believe that pending the results of more biologically intense data analyses, the T3I radiation process does not harm red blood cells, platelets or plasma proteins...**Impression: T3I radiation methodology as used in this protocol, does not harm blood products used for transfusion.**"
>
> Yale Report, page 10 (emphasis in original).

17. That the results and conclusions set forth in the Yale Report establish that the claimed Treatment System is effective to treat a complex fluid [i.e., fresh frozen plasma, platelet concentrates, red blood cells, Adsol® solution, and packed red blood cells] that includes "at least one component that is sensitive to a change in temperature" and that the claimed Treatment System is further "effective to prevent the complex fluid from undergoing a temperature change damaging to the sensitive component."

18. That with reference to the MBT Report, the MBT testing was "designed to provide proof of principle testing phase III and to validate virucidal effectiveness claims for a devise to be used as a virucide," i.e., the claimed Treatment System. "[The testing] determines the potential of the test agent [i.e., the claimed Treatment System] to disinfect blood and blood products contaminated with viruses." [See MBT Report, page 2]

19. That the test procedures and materials used in the MBT testing are set forth at pages 2-8 of the MBT Report. The T3I Hemalight system used in the MBT testing corresponded to the Treatment System referenced above. As set forth in the "Test Evaluation Criteria" at page 8, test sample infected with porcine parvovirus ("PPV") was deemed to be effectively treated by the claimed Treatment System "if there is a 4-5 $\log_{10}$ reduction when test samples are compared to the Process controls of both FFP [fresh frozen plasma] and platelets respectively."

20. That based on the testing reported in the MBT Report, fresh frozen plasma and platelets were effectively treated using the claimed Treatment System. The author of the MBT Report summarized MBT's conclusions as follows:

> When *Porcine parvovirus* (PPV)-spiked fresh frozen plasma (FFP) and platelet bags were exposed to T3I "Hemalight" system [the claimed Treatment System] by the sponsor, T3I "Hemalight" system inactivated $\geq$ 5 $\log_{10}$ of infectious PPV, as compared to the process controls...These conclusions are based on observed data.
>
> MBT Report, page 11.

21. That the results and conclusions set forth in the MBT Report establish that the claimed Treatment System is effective to treat a complex fluid [i.e., fresh frozen plasma and platelets] to inactivate a virus contained therein [i.e., PPV] to achieve a $\geq$ 5 $\log_{10}$ reduction in infectious level as compared to a process control.

22. That when read together, the Yale Report and the MBT Report establish that the claimed Treatment System is capable of virus inactivation [$\geq$ 5 $\log_{10}$ reductions in infectious levels] of complex fluids that are infected, while simultaneously affecting no harm to sensitive components contained in such complex fluid.

23. That the results achieved in complex fluid treatment using the claimed treatment system as described herein are highly advantageous, unexpected, and clearly neither taught nor suggested in the prior art references relied upon in rejecting applicants' pending claims, i.e., the Holbert '934 patent, the Sizer '374 patent, and/or the Nakamura '821 patent.

24. That additional blood-related tests have been performed using treatment systems of the type claimed in the Application. These additional tests have involved a series of bacteria and viral organisms. These tests have been performed with third parties pursuant to confidentiality obligations that do not permit the test results to be disclosed. By way of summary, some of these blood-related test results involving other bacteria and viral organisms have satisfied applicable performance criteria (e.g., $\geq$ 5 $\log_{10}$ reductions in infectious levels without damage to blood constituents) while other test results have failed to satisfy such performance criteria. However, no test results have contradicted or called into question the test results obtained by Yale

and MBT, as reported in the Yale Report and the MBT Report appended hereto as Exhibit A and Exhibit B, respectively.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 8/23/04

BARRY RESSLER

HARTFORD: 621466.01

T3I – Evaluation of Blood Components Irradiated by a Novel Monochromatic Light Source

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Department of Laboratory Medicine

Blood Bank Research and Development Laboratory

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The purpose of this study was to determine the effects, if any, on blood components irradiated by a novel monochromatic light source. Both pooled and individual blood products were aliquoted and subjected to various radiation exposure intensity by T3I engineers at their facility. Various pertinent laboratory assays were used to evaluate possible product changes resulting from the treatment.

Materials and Methods

I. Fresh Frozen Plasma

- Pooled 4 units of in-dated, thawed, fresh frozen plasma into a 2L bag. Final total volume 1179mL.
- Aliquots with volumes ranging from 50mL to 250mL were prepared using sterile docking device and transferred into 150mL transfer bags.
- Products were aliquoted and stored in a refrigerator (1-6°C) until pick up the next morning for treatment by T3I
- Products were irradiated at T3I facility.
- After radiation and return of bags, samples for assay, were removed from product containers using a sampling site coupler and syringe.
- All samples were frozen for batch testing - both initial and 24hr samples. See **TABLE I** for assays performed.
- Automated instrumentation (MLA Electra 1000™ and MLA Electra 1800™) was used for all coagulation time/factor assays.

II. Platelet Concentrates

- Pooled 5 units of four-day-old random donor platelet concentrates into a 1L bag. Mixed well. Total volume was 250mL.
- Aliquoted three (60 mL) samples using sterile docking device into 150mL transfer packs; stored in platelet incubator/shaker.
- The remaining three (20mL) platelet samples (now five days old) were aliquoted just before pickup for treatment.
- Products were irradiated at T3I facility.
- After radiation and return of bags, samples were removed from product containers using a sampling site coupler and syringe.
- All testing was performed same day and 24hrs later. LDH was refrigerated for batch testing. P-Selectin (CD62) was also batched for testing. See **TABLE II** for assays performed.

III. Red Blood Cells

A. Whole Blood

- Whole blood equivalents were prepared by adding ABO compatible, thawed Fresh Frozen Plasma to packed red blood cells (2 weeks old), with a resulting hematocrit ranging from 35 to 40%.
- Aliquots from each individual unit were made using a sterile docking device and transferred into 150mL transfer bags. Aliquot volumes ranged from 25 to 104mL and hematocrits ranged from 29%-68%. The latter was due to difficulties frequently

encountered in mixing blood. One must mix well but avoid high shear stresses which would induce mechanical hemolysis.

- Products were irradiated at T3I facility
- After radiation and return of bags, samples were removed from product containers using a sampling site coupler and syringe.
- Hemoglobin and hematocrit were performed the same day and 24hrs later. All other samples were frozen and batched for testing. Plasma hemoglobins were also sampled at 48hours. See **TABLE III** for assays performed.

B. ADSOL

- 3 individual units of Adsol RBC's (5-6 weeks old) with hematocrit ranging from 52-66% were used.
- Aliquots from each individual unit were made using a sterile docking device and transferred into 150mL transfer bags. Volumes ranged from 18 to 104mL.; hematocrits from 52-66%.
- Products were irradiated at T3I facility
- After radiation and return of bags, samples were removed from product containers using a sampling site coupler and syringe.
- Hemoglobin and Hematocrit were performed the same day and 24hrs later. Plasma hemoglobins were also sampled at 48hours
- All other samples were frozen and batched for testing. See **TABLE IV** for assays performed.

C. Packed Red Cells

- 3 individual units of packed red cells (1 week old) with a hematocrit ranging from 73-80% were used.
- Aliquots from each individual unit were made using sterile docking device and transferred into 150mL transfer bags. Volumes range from 15mL to 75mL; hematocrits from 73-80%.
- Products were irradiated at T3I facility.
- After radiation and return of bags, samples were removed from product containers using a sampling site coupler and syringe.
- Due to minimal plasma volume available from packed RBC's, all plasma supernatants removed, were diluted with equal volumes of normal saline to obtain adequate samples for K^+, LDH, and plasma hemoglobin testing. Final results were corrected for this 1:1 dilution.
- Hemoglobin and hematocrit were performed the same day and 24hrs later. All other samples were frozen and batched for testing. Plasma hemoglobins were also sampled at 48hours.
- See **Table V** for assays performed.

IV. Assay Measurement

- Hemoglobin and hematocrit measured using BAKER system 9110 hematology analyzer.
- LDH determined using HITACHI automatic analyzer 747-200.
- K^+ determined using BECKMAN CX3 DELTA.

- ATP and 2,3- DPG determined using SIGMA Diagnostics kit spectrophotometh ic assay.
- Plasma Hemoglobin evaluated using Wians' direct spectrophotometric scannin procedure for quantitating plasma hemoglobin (HITACHI U-2000 Spectrophotometer).
- **SOP's (Standard Operating Procedures) available on request for all proce lures**

RESULTS

Results seen for Fresh Frozen Plasma **(Table 1)** show that there was no clinically significant difference between results for the control aliquot (C-2) and the 9 test (irradiated products, C-1 and C-3 – C-10). Similarly, at 24-hours there was no clini ally significant difference between the C-2 control of 13.6 secs for the prothrombin time (PT) and all results for the high, medium and low irradiated products that were stored for 24 hours. Similarly, for PTT (partial thromboplastin time), fibrinogen and Factor VIII, the levels of Factor VIII are slightly lower 24 hours after radiation and the PT and PTT clotting times are slightly longer after 24 hours of storage, versus immediately after irradiation on day 1. These changes are due to storage effect and not due to radiation.

Impression: No clinically significant effect due to irradiation seen on the aliquots of fresh frozen plasma irradiated and stored for 24 hours as performed under the experimental conditions used.

Results for Platelet Concentrates (**Table 2**), showed that there were no significant changes between control platelet count and test platelet count on the day of irradiation nor were there clinically significant differences for platelet count for platelets stored for 24 hours control or test. The same is true for CD62, morphology score, LDH, pH, pO_2, pCO_2 or bicarbonate (HC03). For the osmotic recovery assay, there is a slight suggestion that osmotic recovery may have decreased 24 hours after irradiation in the test group compared to control which was the same, 50% on day 0 and on day 1 after radiation for control. This trend, however, should likely have been re-enforced by additional in vitro results which did not show a similar trend. There appears to be no substantial change in platelet count or platelet functionality after radiation at high, medium or low treatment protocol levels. Additional studies would be indicated for indated platelets stored for a full 5 days post irradiation.

Impression: There is no clinically significant effect on platelets treated with high, low or medium doses of radiation and then stored for up to 24 hours.

For whole blood, (**Table 3**) results show a wide variation in the levels of hemoglobin in aliquots of units WB A1, WB A2. Aliquots from other units such as WB A6, WB A4, are much more consistent. In addition, there are several examples of a lack of consistency in aliquot hemoglobin concentrations and hemoglobin between Day 0 and Day 1 samples. For example, hct (hematocrit) for WB B7 at day 1 is different from WB B7 at 24 hours post. This is mostly likely due to sampling issues as described above. The C5 unit series, did not show such changes. This was most likely an error in

processing rather than an actual effect of radiation. Hematocrit follows similarly w th hemoglobin, as does LDH and potassium levels. For LDH the highest levels such as WB A1, WB A2, correlate with the highest levels of hemoglobin. Similarly, WB A1, ar d A2, at 24 hours are also high. WB B1, and WB B2, are also high for LDH, 662 and 549 but they, too, are also associated with the higher hemoglobin levels of 17.3, 17.3. Simi arly, potassium is elevated for the WB B1, WB B2, and the WB A1, and WB A2. No su h analyte elevation were noted in the WB "C group" which had much more uniform ai iquot distribution. For ATP there was no clinically significant difference between the pre and the post for any of the samples evaluated. Plasma hemoglobin was similarly elevate l in the A1, A2 and B1, B2, groups. The plasma hemoglobin level for WB A2, of 8.5 is ow and is most likely explained as aliquot maldistribution rather than an effect of irradia tion. No problems were seen with plasma hemoglobin determination on the C5 group. Pe vent hemolysis paralleled that of plasma hemoglobin and the 48-hour hemoglobin values showed no specific changes. All changes seen were likely due to storage.

Impression: For whole blood, radiation at different intensities produced no evidence of radiation-induced damage. Rather, the damage that was seen was attributable to sampling error and time of storage.

Table 4, Adsol: Results for Adsol where the plasma has been replaced with crystallo d solution were similar. However, Adsol aliquots were more consistent in that the hemoglobins were much similar between control and test, for all groups, A, B,and C. The LDHs, potassiums, plasma hemoglobins and percent hemolysis were also simil r

among the aliquots of all three Adsol units tested. ATP levels were somewhat lower than were seen for whole blood which could relate to the storage age of the product but the differences in ATP levels were not clinically significantly different. Forty-eight hour plasma hemoglobin levels were also all comparable.

Impression: For Adsol, radiation at different intensities produced no evidence of radiation-induced damage.

For Packed Red Blood Cells (**Table 5**), the results of the aliquoting was consistent as seen by similar values for plasma hemoglobin, hematocrit, LDH and potassium. ATPs again showed no clinically significant differences. No evidence of irradiation-induced damage was seen, no trends were seen, no clinically significant changes were seen.

Impression: Packed red cells are not affected by T3I radiation using the protocols performed under this trial.

Discussion:

The purpose of this pilot research project was to evaluate the effect of varying intensities of monochromatic light radiation on transfusable blood products. These components include thawed fresh frozen plasma, platelet concentrates and three types of red cells with varying hematocrits. Results showed changes related to storage time and distribution of sample but not, on a consistent basis, due to T3I radiation. Had radiation been a problem markedly abnormal results should have been seen in various assays. Such multi-assay abnormalities were not seen. Changes induced by radiation should not be subtle. Those

analyte results that were out of the ordinary or appeared erroneous were isolated and were not corroborated by similar changes in other analytes.

Our final impression is that there is no consistent trend. While admitting that the sample population is small, under the conditions evaluated there appears to be no clinically significant damage inflicted on blood products due to T3I-induced irradiation. Consistent changes due to storage of blood products for 24-48 hours post radiation, however, were seen. It would appear that there are no reasons not to proceed to the second phase of testing and proceed to more intense product evaluation . Thus, we believe that pending the results of more biologically intense data analyses, the T3I radiation process does not harm red blood cells, platelets or plasma proteins. Further studies will be done in the more intensive Phase II studies.

Impression: T3I radiation methodology as used in this protocol, does not harm blood products used for transfusion.

Table I - Fresh Frozen Plasma Results

ALIQUOT POST	TREATMENT	PT	PTT	FIBRINOGEN	FACTOR VIII (%)
C-2 97ml CONTROL	N/A	*13.1*	*35.4*	*227*	*67*
C-1 251ml	MED	13.0	35.4	218	65
C-3 101ml	MED	13.0	35.6	215	64
C-4 101ml	HI	13.0	35.8	223	65
C-5 57ml	LOW	12.9	35.7	242	65
C-6 51ml	MED	13.2	35.6	224	64
C-7 50ml	HIGH	13.0	35.7	221	63
C-8 51ml	HIGH	13.1	36.3	207	60
C-9 50ml	HIGH	13.0	35.5	225	63
C-10 50ml	LOW	13.0	34.9	222	66
Mean		**13.0**	**35.6**	**222**	**64**
1 SD		**0.08**	**0.37**	**9.4**	**1.8**

ALIQUOT 24Hr.	TREATMENT	PT	PTT	FIBRINOGEN	FACTOR VIII
C-2 97ml CONTROL	N/A	*13.6*	*36.5*	*217*	*61*
C-1 251ml	MED	13.7	36.2	211	59
C-3 101ml	MED	13.7	36.7	216	61
C-4 101ml	HI	13.8	37.0	219	61
C-5 57ml	LOW	13.7	36.6	227	60
C-6 51ml	MED	13.6	36.4	233	62
C-7 50ml	HIGH	13.6	36.6	229	61
C-8 51ml	HIGH	13.6	36.0	232	61
C-9 50ml	HIGH	13.8	36.7	222	60
C-10 50ml	LOW	13.5	36.0	233	63
Mean		**13.7**	**36.5**	**225**	**61**
1 SD		**0.10**	**0.34**	**8**	**1**

Table II -Platelet Concentrate Results

ALIQUOT POST	TREATMENT	Platelet ($x10^3$/uL)	CD62 (% activated)	Morphology Score (Max = 800)	Osmotic Recovery (%)	LDH (% released)	pH (25° C)	pO_2 (mmHg)	pCO_2 (mmHg)	HCO_3 (mmol/L)
B-1 CONTROL 57ml	*N/A*	*1540*	*34.4*	*587*	*50*	*6.3*	*7.13*	*77*	*21*	*8.6*
B-2 60ml	HIGH	1520	41.6	604	59	7.0	7.11	72	23.0	8.9
B-3 60ml	LOW	1470	40.3	622	56	6.6	7.11	66	23.0	8.9
B-4 20ml	HIGH	1520	42.0	596	47	7.3	7.27	106	12.0	6.5
B-5 20ml	MED	1370	39.0	602	50	7.1	7.27	95	12.0	6.6
B-6 20ml	LOW	1370	38.2	622	57	6.8	7.29	95	11.0	6.5
MEAN		1450	40.2	609	54	7.0	7.21	86.8	16.2	7.6
1SD		75.8	1.6	12	5.1	0.3	0.09	17.0	6.2	1.3

Note: WBC ($x\ 10^3$/uL) not reported due to negligible value with leukoreduced products

ALIQUOT 24Hr.	TREATMENT	Platelet ($x10^3$/uL)	CD62 (% activated)	Morphology Score (Max = 800)	Osmotic Recovery (%)	LDH (% released)	pH (25° C)	pO_2 (mmHg)	pCO_2 (mmHg)	HCO_3 (mmol/L)
B-1 CONTROL 57ml	*N/A*	*1450*	*42.1*	*605*	*50*	*17.1*	*7.05*	*69*	*22.3*	*7.7*
B-2 60ml		1470	44.2	580	45	17.3	7.02	68	24.3	7.8
B-3 60ml		1410	41.7	568	45	16.6	7.03	63	23.3	7.7
B-4 20ml		1310	47.6	556	43	18.4	7.08	100	12.9	4.7
B-5 20ml		1400	45.8	546	43	18.3	7.09	92	13.2	4.9
B-6 20ml		1370	46.9	509	45	18.1	7.09	90	12.9	4.8
MEAN		1392	45	552	44	18	7.06	83	17	6.0
1SD		58.5	2.4	27	1.1	0.8	0.03	16.1	5.9	1.6

Table III - Whole Blood Results

ALIQUOT	TREAT-MENT	WB Hgb (g/dL)	WB HCT (%)	LDH (U/L)	K^+ (mmol/L)	ATP umol/g Hb	2,3-DPG umol/g Hb	Plasma Hgb (mg/dL)	% Hemo-lysis		
WB A5 CTRL 76ml	***N/A***	***11.2***	***35***	***504***	***14.0***	***3.76***		***10.2***	***0.06***		
WB A1 39ml	B	21.7	68	1292	31.4	N/T		76.7	*0.11*		
WB A2 41ml	A	20.0	65	1080	26.6	N/T		8.5	*0.01*		
WB A3 38ml	C	18.2	57	878	23.3	N/T		34.1	*0.08*		
WB A4 39ml	D	16.1	50	781	19.8	N/T		35.9	*0.11*		
WB A6 76ml	A	9.3	29	463	12.3	3.59		5.5	*0.04*		
WB A7 145ml	A	10.5	31	439	11.6	N/T		4.4	*0.03*		
MEAN	**N/A**	**16.0**	**50.0**	**819**	**20.8**	**N/A**		**27.5**	**0.07**		
1SD	**N/A**	**5.1**	**16.7**	**334**	**7.9**	**N/A**		**28.0**	**0.04**		

ALIQUOT POST 24hr	TREAT-MENT	WB Hgb (g/dL)	WB HCT (%)	LDH (U/L)	K^+ (mmol/L)	ATP umol/g Hb	2,3-DPG umol/g Hb	Plasma Hgb (mg/dL)	% Hemo-lysis	Plasma Hgb(mg/dL) - *48 hr*	% Hemo-lysis
WB A5 CTRL 76ml	***N/A***	***11.5***	***35***	***594***	***14.8***	***3.59***		***6.7***	***0.04***	***30.6***	***0.17***
WB A1 39ml	B	21.4	68	1480	33.7	N/T		83	*0.12*	76.7	*0.11*
WB A2 41ml	A	20.1	63	1209	29.0	N/T		56.4	*0.10*	71.1	*0.13*
WB A3 38ml	C	18.0	57	1020	24.9	N/T		20.7	*0.05*	22.3	*0.05*
WB A4 39ml	D	16.0	51	943	21.2	N/T		42.3	*0.13*	50.9	*0.16*
WB A6 76ml	A	10.4	31	482	12.9	3.08		6.6	*0.04*	8.1	*0.05*
WB A7 145ml	A	8.7	26	446	12.3	N/T		4.5	*0.04*	5.9	*0.05*
MEAN	**N/A**	**15.8**	**49.3**	**930**	**22.3**	**N/A**		**35.6**	**0.08**	**39.2**	**0.1**
1SD	**N/A**	**5.2**	**17.2**	**406**	**8.6**	**N/A**		**30.8**	**0.0**	**31.4**	**0.05**

ALIQUOT	TREAT-MENT	WB Hgb (g/dL)	WB HCT (%)	LDH (U/L)	K^+ (mmol/L)	ATP umol/g Hb	2,3-DPG umol/g Hb	Plasma Hgb (mg/dL)	% Hemo-lysis		
WB B5 CTRL 74ml	***N/A***	***11.2***	***35***	***332***	***11.4***	***4.96***		***10.8***	***0.06***		
WB B1 35ml	B	17.3	54	662	19.2	N/T		54.3	*0.14*		
WB B2 38ml	A	17.3	54	549	18.0	N/T		5.7	*0.02*		
WB B3 34ml	C	16.8	52	511	17.5	N/T		30.5	*0.09*		
WB B4 40ml	D	15.8	49	487	15.6	N/T		23.9	*0.08*		
WB B6 75ml	A	10.0	30	351	11.4	4.76		10.2	*0.07*		
WB B7 141ml	A	10.1	30	333	10.3	N/T		8.7	*0.03*		
MEAN	**N/A**	**15.6**	**48.2**	**482**	**15.3**	**N/A**		**22.2**	**0.07**		
1SD	**N/A**	**2.8**	**9.1**	**124**	**3.7**	**N/A**		**18.4**	**0.05**		

Table III - Whole Blood Results

ALIQUOT POST 24hr	TREAT-MENT	WB Hgb (g/dL)	WB HCT (%)	LDH (U/L)	K^+ (mmol/L)	ATP umol/g Hb	2,3-DPG umol/g Hb	Plasma Hgb (mg/dL)	% Hemo-lysis	Plasma Hgb(mg/dL) - *48 hr*	% Hemo-lysis
WB B5 CTRL 74ml	***N/A***	***11.3***	***34***	***373***	***12.5***	***4.54***		***12.9***	***0.08***	***24.8***	***0.14***
WB B1 35ml	B	17.3	53	700	20.5	N/T		66.7	*0.18*	70.6	*0.19*
WB B2 38ml	A	17.0	54	625	20.1	N/T		38.5	*0.10*	94.2	*0.25*
WB B3 34ml	C	16.6	52	601	19.1	N/T		42.1	*0.12*	48.8	*0.14*
WB B4 40ml	D	15.9	49	555	17.2	N/T		38.2	*0.12*	52.8	*0.17*
WB B6 75ml	A	10.0	30	335	11.4	4.49		10	*0.07*	13.3	*0.09*
WB B7 141ml	A	8.7	27	321	10.7	N/T		8.1	*0.07*	8.6	*0.07*
MEAN	**N/A**	**14.3**	**44.2**	**523**	**16.5**	**N/A**		**33.9**	**0.11**	**48.1**	**0.2**
1SD	**N/A**	**3.8**	**12.3**	**158**	**4.4**	**N/A**		**22.0**	**0.04**	**32.9**	**0.07**

ALIQUOT	TREAT-MENT	WB Hgb (g/dL)	WB HCT (%)	LDH (U/L)	K^+ (mmol/L)	ATP umol/g Hb	2,3-DPG umol/g Hb	Plasma Hgb (mg/dL)	% Hemo-lysis		
WB C5 CTRL 75ml	***N/A***	***13.2***	***40***	***644***	***14.3***	***4.36***		***23.2***	***0.11***		
WB C1 39ml	B	13.1	40	634	14.0	N/T		12.3	*0.06*		
WB C2 41ml	A	13.5	42	656	14.3	N/T		26.4	*0.11*		
WB C3 38ml	C	13.7	41	674	14.5	N/T		30.7	*0.13*		
WB C4 41ml	D	13.7	41	658	14.3	N/T		37	*0.16*		
WB C6 74ml	A	13.2	40	639	14.2	3.83		24.9	*0.11*		
WB C7 141ml	A	13.9	43	655	14.4	N/T		19.6	*0.08*		
MEAN	**N/A**	**13.5**	**41.2**	**653**	**14.3**	**N/A**		**25.2**	**0.11**		
1SD	**N/A**	**0.3**	**1.2**	**14**	**0.2**	**N/A**		**8.6**	**0.04**		

ALIQUOT POST 24hr	TREAT-MENT	WB Hgb (g/dL)	WB HCT (%)	LDH (U/L)	K^+ (mmol/L)	ATP umol/g Hb	2,3-DPG umol/g Hb	Plasma Hgb (mg/dL)	% Hemo-lysis	Plasma Hgb(mg/dL) - *48 hr*	% Hemo-lysis
WB C5 CTRL 75ml	***N/A***	***13.6***	***42***	***677***	***15.0***	***3.54***		***32.1***	***0.14***	***41.8***	***0.18***
WB C1 39ml	B	12.9	39	686	14.6	N/T		42.4	*0.20*	51.4	*0.24*
WB C2 41ml	A	13.6	41	682	15.0	N/T		29.8	*0.13*	42.6	*0.18*
WB C3 38ml	C	13.1	41	713	15.0	N/T		81.2	*0.37*	47.2	*0.21*
WB C4 41ml	D	13.5	42	807	15.0	N/T		37.9	*0.16*	57.2	*0.25*
WB C6 74ml	A	12.9	40	679	15.0	3.76		27.3	*0.13*	29.4	*0.14*
WB C7 141ml	A	13.3	42	670	15.1	N/T		23.1	*0.10*	83.3	*0.36*
MEAN	**N/A**	**13.2**	**40.8**	**706**	**15.0**	**N/A**		**40.3**	**0.2**	**51.9**	**0.2**
1SD	**N/A**	**0.3**	**1.2**	**51**	**0.2**	**N/A**		**21.3**	**0.1**	**18.1**	**0.08**

Table IV - Adsol Results

ALIQUOT POST	TREAT-MENT	ADS Hgb (g/dL)	ADS HCT (%)	LDH (U/L)	K^+ (mmol/L)	ATP umol/g Hb	2,3-DPG umol/g Hb	Plasma Hgb (mg/dL)	% Hemo-lysis		
ADS A5 CONTROL 32ml	*N/A*	*16.7*	*53*	*206*	*41.9*	2.22		*45*	*0.13*		
ADS A1 40ml	A	16.9	53	187	42.0	N/T		77.8	*0.22*		
ADS A2 27ml	B	16.7	53	199	42.2	N/T		85.5	*0.24*		
ADS A3 43ml	C	16.9	53	149	42.4	N/T		60.7	*0.17*		
ADS A4 39ml	D	16.9	53	159	42.1	N/T		62	*0.17*		
ADS A6 35ml	A	16.8	53	143	41.9	2.11		63.4	*0.18*		
ADS A7 88ml	A	16.6	53	142	41.6	N/T		64	*0.18*		
MEAN		**16.8**	**53.0**	**163.2**	**42.0**	**NA**		**68.9**	**0.2**		
1SD		**0.1**	**0.0**	**24.2**	**0.3**	**NA**		**10.2**	**0.0**		

ALIQUOT POST 24hr	TREAT-MENT	ADS Hgb (g/dL)	ADS HCT (%)	LDH (U/L)	K^+ (mmol/L)	ATP umol/g Hb	2,3-DPG umol/g Hb	Plasma Hgb (mg/dL)	% Hemo-lysis	Plasma Hgb(mg/dL) - *48 hr*	% Hemo-lysis
ADS A5 CONTROL 32ml	*N/A*	*16.7*	*52*	*240*	*42.4*	*2.14*		*73.5*	*0.21*	*48.6*	*0.14*
ADS A1 40ml	A	16.2	53	299	42.5	N/T		95.6	*0.28*	25.6	*0.07*
ADS A2 27ml	B	16.5	51	339	42.6	N/T		83.5	*0.25*	76.3	*0.23*
ADS A3 43ml	C	16.6	53	142	40.6	N/T		108.9	*0.31*	88.4	*0.25*
ADS A4 39ml	D	17.1	53	136	40.1	N/T		66.7	*0.18*	85.8	*0.24*
ADS A6 35ml	A	17.8	57	142	40.6	2.10		73.2	*0.18*	126.7	*0.31*
ADS A7 88ml	A	17.3	55	107	41.1	N/T		72.0	*0.19*	82.9	*0.22*
MEAN		**16.9**	**53.7**	**194.2**	**41.3**	**NA**		**83.3**	**0.2**	**81.0**	**0.2**
1SD		**0.6**	**2.1**	**98.4**	**1.1**	**NA**		**16.2**	**0.1**	**32.4**	**0.1**

ALIQUOT POST	TREATMENT	ADS Hgb (g/dL)	ADS HCT (%)	LDH (U/L)	K^+ (mmol/L)	ATP umol/g Hb	2,3-DPG umol/g Hb	Plasma Hgb (mg/dL)	% Hemo-lysis		
ADS B5 CONTROL 52ml	*N/A*	*20.9*	*66*	*263*	*43.3*	*2.35*		*101.9*	*0.17*		
ADS B1 29ml	A	19.8	62	295	42.6	N/T		64.7	*0.12*		
ADS B2 30ml	B	21.2	67	280	42.6	N/T		102.8	*0.16*		
ADS B3 29ml	C	20.7	69	287	39.7	N/T		90.3	*0.14*		
ADS B4 30ml	D	21.1	65	275	43.1	N/T		74.4	*0.12*		
ADS B6 54ml	A	20.8	66	259	43.1	2.14		93.3	*0.15*		
ADS B7 80ml	A	20.8	66	245	43.4	N/T		57.8	*0.09*		
MEAN		**20.7**	**65.8**	**273.5**	**42.4**	**NA**		**80.6**	**0.1**		
1SD		**0.5**	**2.3**	**18.5**	**1.4**	**NA**		**17.7**	**0.0**		

ALIQUOT POST 24hr	TREATMENT	ADS Hgb (g/dL)	ADS HCT (%)	LDH (U/L)	K^+ (mmol/L)	ATP umol/g Hb	2,3-DPG umol/g Hb	Plasma Hgb (mg/dL)	% Hemo-lysis	Plasma Hgb(mg/dL) - *48 hr*	% Hemo-lysis

Table IV - Adsol Results

ADS B5 CONTROL 52ml	N/A	20.8	66	311	44.1	2.24		72.8	0.12	58.0	0.09
ADS B1 29ml	A	21	67	415	43.1	N/T		82.3	0.13	46.0	0.07
ADS B2 30ml	B	20.6	66	344	43.6	N/T		71.1	0.12	52.8	0.09
ADS B3 29ml	C	20.3	64	451	44.2	N/T		55.4	0.10	65.4	0.12
ADS B4 30ml	D	20.3	64	387	44.1	N/T		75.7	0.13	75.0	0.13
ADS B6 54ml	A	20.8	67	304	43.8	2.37		62.3	0.10	46.2	0.07
ADS B7 80ml	A	20.7	65	282	44.1	N/T		42	0.07	68.5	0.12
MEAN		20.6	65.6	363.8	43.8	NA		64.8	0.1	59.0	0.1
1SD		0.3	1.4	65.5	0.4	NA		14.7	0.0	12.3	0.0

ALIQUOT	TREATMENT	ADS Hgb (g/dL)	ADS HCT (%)	LDH (U/L)	K^+ (mmol/L)	ATP umol/g Hb	2,3-DPG umol/g Hb	Plasma Hgb (mg/dL)	% Hemo-lysis		
ADS C5 CONTROL 54ml	N/A	16.9	53	145	39.8	3.18		67.1	0.19		
ADS C1 25ml	A	17.3	53	183	39.9	N/T		43.6	0.12		
ADS C2 27ml	B	16.9	53	161	39.9	N/T		17.4	0.05		
ADS C3 26ml	C	17	53	157	39.8	N/T		67.4	0.19		
ADS C4 26ml	D	17.3	53	149	40.0	N/T		69.0	0.19		
ADS C6 49ml	A	17.0	53	157	40.0	3.23		71.0	0.20		
ADS C7 104ml	A	17.1	53	131	40.3	N/T		49.4	0.14		
MEAN		17.1	53.0	156.3	40.0	NA		53.0	0.1		
1SD		0.2	0.0	16.9	0.2	NA		20.6	0.1		

ALIQUOT POST 24hr	TREATMENT	ADS Hgb (g/dL)	ADS HCT (%)	LDH (U/L)	K^+ (mmol/L)	ATP umol/g Hb	2,3-DPG umol/g Hb	Plasma Hgb (mg/dL)	% Hemo-lysis	Plasma Hgb(mg/dL) - 48 hr	% Hemo-lysis
ADS C5 CONTROL 54ml	N/A	17	52	174	40.5	3.36		84.1	0.24	81.8	0.23
ADS C1 25ml	A	16.8	52	242	39.9	N/T		87.2	0.25	62.0	0.18
ADS C2 27ml	B	17	51	244	40.4	N/T		128.9	0.37	71.8	0.21
ADS C3 26ml	C	16.8	52	256	40.1	N/T		76.8	0.22	69.1	0.20
ADS C4 26ml	D	16.8	52	209	40.4	N/T		83.5	0.24	81.6	0.23
ADS C6 49ml	A	16.8	53	225	40.5	3.10		95.8	0.27	73.3	0.21
ADS C7 104ml	A	16.8	53	164	40.5	N/T		43.6	0.12	79.3	0.22
MEAN		16.8	52.2	223.3	40.3	NA		86.0	0.2	72.9	0.2
1SD		0.1	0.8	33.4	0.2	NA		27.7	0.1	7.1	0.0

Table V - Packed RBC's Results

ALIQUOT	TREAT-MENT	PRBC Hgb (g/dL)	PRBC HCT (%)	LDH (U/L)	K^+ (mmol/L)	ATP umol/g Hb	2,3-DPG umol/g Hb	Plasma Hgb (mg/dL)	% Hemo-lysis		
PRC A5 CONTROL 36ml	*N/A*	*24.3*	*73*	*860*	*27.8*	*1.91*		*124.2*	*0.14*		
PRC A1 33ml	A	23.0	72	1164	26.2	N/T		147.8	0.18		
PRC A2 20ml	B	23.4	72	954	26.8	N/T		249.1	0.30		
PRC A3 17ml	C	23.3	73	946	26.8	N/T		201.1	0.23		
PRC A4 15ml	D	24.0	73	1230	27.4	N/T		187.5	0.21		
PRC A6 36ml	A	22.9	73	926	27.2	3.04		40.8	0.05		
PRC A7 74ml	A	23.2	73	882	28.2	N/T		154.4	0.18		
MEAN		**23.3**	**72.7**	**1017.0**	**27.1**	NA		**163.5**	**0.19**		
1SD		0.4	0.5	143.2	0.7	NA		70.3	0.08		
ALIQUOT POST 24hr	**TREAT-MENT**	**PRBC Hgb (g/dL)**	**PRBC HCT (%)**	**LDH (U/L)**	**K^+ (mmol/L)**	**ATP umol/g Hb**	**2,3-DPG umol/g Hb**	**Plasma Hgb (mg/dL)**	**% Hemo-lysis**	**Plasma Hgb(mg/dL) - *48 hr***	**% Hemo-lysis**
PRC A5 CONTROL 36ml	*N/A*	*24.3*	*76*	*1040*	*29.6*	*2.68*		*188.8*	*0.19*	*154.2*	*0.15*
PRC A1 33ml	A	23.9	74	1256	26.4	N/T		153.6	0.17	165.6	0.18
PRC A2 20ml	B	24.7	75	960	27.6	N/T		188.5	0.19	215	0.22
PRC A3 17ml	C	24.3	75	960	27.2	N/T		22.7	0.02	N/T	N/T
PRC A4 15ml	D	24.3	76	1336	26.4	N/T		183.8	0.16	N/T	N/T
PRC A6 36ml	A	24.5	76	1016	28	2.71		31.6	0.03	28.1	0.03
PRC A7 74ml	A	24.4	76	928	28.8	N/T		248.6	0.24	84.3	0.08
MEAN		**24.4**	**75**	**1076**	**27.4**	NA		**134.8**	**0.14**	**123.3**	**0.13**
1SD		0.3	1	175	0.9	NA		89.7	0.09	83.2	0.09
ALIQUOT	**TREAT-MENT**	**PRBC Hgb (g/dL)**	**PRBC HCT (%)**	**LDH (U/L)**	**K^+ (mmol/L)**	**ATP umol/g Hb**	**2,3-DPG umol/g Hb**	**Plasma Hgb (mg/dL)**	**% Hemo-lysis**		
PRC B5 CONTROL 39ml	*N/A*	*26.3*	*79*	*1772*	*53.6*	*1.86*		*150.7*	*0.12*		
PRC B1 19ml	A	26.5	77	2758	51.2	N/T		136.6	0.12		
PRC B2 19ml	B	25.4	79	1684	49.2	N/T		107.5	0.09		
PRC B3 19ml	C	26.3	77	1690	51.4	N/T		60.3	0.05		
PRC B4 20ml	D	25.2	76	1834	53	N/T		141.8	0.14		
PRC B6 39ml	A	26.0	79	1620	50.4	0.97		106	0.09		
PRC B7 74ml	A	26.1	N/T	1704	53.6	N/T		72.2	N/T		
MEAN		**25.9**	**77.6**	**1915.0**	**51.5**	NA		**104.1**	**0.1**		
1SD		0.5	1.3	424.9	1.6	NA		33.0	0.0		

Table V - Packed RBC's Results

ALIQUOT 24hr	TREAT-MENT	PRBC Hgb (g/dL)	PRBC HCT (%)	LDH (U/L)	K^+ (mmol/L)	ATP umol/g Hb	2,3-DPG umol/g Hb	Plasma Hgb (mg/dL)	% Hemo-lysis	Plasma Hgb(mg/dL) - *48 hr*	% Hemo-lysis
PRC B5 CONTROL 39m	*N/A*	*26.0*	*80*	*1968*	*55.2*	*2.21*		*119.6*	*0.09*	*141.5*	*0.11*
PRC B1 19ml	A	26.1	79	4284	58.4	N/T		260.1	0.21	47.4	0.04
PRC B2 19ml	B	26.0	81	160	41.6	N/T		170.5	0.13	105.2	0.08
PRC B3 19ml	C	26.3	80	2544	54.4	N/T		118.6	0.09	26.4	0.02
PRC B4 20ml	D	26.1	78	1736	48	N/T		69.7	0.06	206.3	0.17
PRC B6 39ml	A	25.5	78	1972	56.8	2.09		166.5	0.14	144	0.12
PRC B7 74ml	A	26.1	80	1866	56.8	N/T		74.1	0.06	143.8	0.11
MEAN		**26.0**	**79.3**	**2090.3**	**52.7**	NA		**143.3**	**0.1**	**112.2**	**0.1**
1SD		0.3	1.2	1330.2	6.5	NA		71.7	0.1	67.0	0.1

ALIQUOT	TREAT-MENT	PRBC Hgb (g/dL)	PRBC HCT (%)	LDH (U/L)	K^+ (mmol/L)	ATP umol/g Hb	2,3-DPG umol/g Hb	Plasma Hgb (mg/dL)	% Hemo-lysis
PRC C5 CONTROL 34m	*N/A*	*25.6*	*82*	*1066*	*51.4*	*2.22*		*169.5*	*0.12*
PRC C1 18ml	A	24.4	73	1648	42.6	N/T		164.6	0.18
PRC C2 19ml	B	25.7	78	1376	50.4	N/T		177.4	0.15
PRC C3 24ml	C	25.7	78	1130	48.2	N/T		337.8	0.29
PRC C4 19ml	D	26.2	80	1298	51.4	N/T		198.9	0.15
PRC C6 40ml	A	26.3	81	1256	51.8	2.16		56.8	0.04
PRC C7 75ml	A	26.0	80	1090	52.4	N/T		162.8	0.13
MEAN		**25.7**	**78**	**1300**	**49.5**	NA		**183.1**	**0.16**
1SD		0.7	3	201	3.7	NA		90.5	0.08

ALIQUOT 24hr	TREAT-MENT	PRBC Hgb (g/dL)	PRBC HCT (%)	LDH (U/L)	K^+ (mmol/L)	ATP umol/g Hb	2,3-DPG umol/g Hb	Plasma Hgb (mg/dL)	% Hemo-lysis	Plasma Hgb(mg/dL) - *48 hr*	% Hemo-lysis
PRC C5 CONTROL 34m	*N/A*	*25.8*	*78*	*1578*	*51.6*	*2.43*		*185.6*	*0.16*	*142.1*	*0.12*
PRC C1 18ml	A	24.1	74	3798	47.2	N/T		40.7	0.04	60.6	0.07
PRC C2 19ml	B	25.6	79	1624	45.8	N/T		185	0.15	145	0.12
PRC C3 24ml	C	25.3	78	1638	44.8	N/T		204.1	0.18	22.5	0.02
PRC C4 19ml	D	24.7	76	1614	49.2	N/T		179.9	0.17	187.8	0.18
PRC C6 40ml	A	25.4	78	1558	50.0	2.58		200.4	0.17	138.1	0.12
PRC C7 75ml	A	25.5	78	1202	47.0	N/T		213.4	0.18	154.7	0.13
MEAN		**25.1**	**77**	**1906**	**47.3**	NA		**170.6**	**0.2**	**118.1**	**0.11**
1SD		0.6	2	942	2.0	NA		64.8	0.1	62.9	0.08

MICROBIOTEST, INC
The Microbiology and Virology Laboratory

MicroBioTest Protocol

EVALUATION OF T^{3}I PATHOGEN INACTIVATION SYSTEM

Using *Porcine parvovirus*

Prepared for
TRITON THALASSIC TECHNOLOGIES INC.
241 Ethan Allen Highway
Ridgefield, CT 06877

REDACTED

*Accredited by the Council for Antimicrobial Quality in Phase I of its laboratory Accreditation Program for GLP compliance and data integrity.

CAQ Council for Antimicrobial Quality

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OBJECTIVE:

This test is designed to provide proof of principle testing phase III and to validate virucidal effectiveness claims for a devise to be used as a virucide. It determines the potential of the test agent to disinfect blood and blood products contaminated with viruses. The test is designed to simulate consumer use and follows the procedure outlined in the American Society for Test Materials (ASTM) test method designated E 1052-96, entitled "Standard Test Method for Efficacy of Antimicrobial Agents Against Viruses in Suspension." The study will be conducted under FDA GLP regulations 21 CFR §58 and the data generated will be used by the sponsor for internal purposes.

TESTING CONDITIONS:

Porcine parvovirus (PPV) spiked blood and blood products will be irradiated using the T^3I apparatus operated at room temperature. T^3I technicians will operate the machine. After the exposure period (as determined by T^3I), the sample will be analyzed for the presence of any residual infectious PPV. Results will be recorded as log_{10}-virus inactivation.

MATERIALS:

A. Test, control and reference substances will be supplied by the sponsor of the study (see last page).

The T^3I apparatus will be delivered and installed by T^3I technicians. MicroBioTest will provide an area with controlled access. MicroBioTest will provide water and electrical utilities. Safety protocols (PPE, training, etc.) will be discussed by T^3I and the lab and mutually agreed to prior to initiation of the test.

Blood components will be supplied by a blood bank (T^3I has made arrangement with Biological Specialty Laboratories through Dr. Steve Diamond to deliver the blood products as and when required by MicroBioTest).

The lab will aliquot blood products into the process bags supplied by T³I. These bags have standard, closable, openings for filling, spiking and post treatment sampling. Only T³I personnel will handle the bags during the irradiation process step. Post-test, virology lab will analyze the samples and the remaining samples will be discarded in a manner that meets the approval of the safety officer.

B. Materials supplied by MicroBioTest, Inc., including, but not limited to:

1. Challenge virus as requested by the sponsor of the study: Porcine parvovirus (PPV).

2. Host cell line: Appropriate for the virus used.

3. Laboratory equipment and supplies.

4. Media and reagents:

   a. Appropriate cell culture media.
   b. Appropriate buffered saline solution.
   c. Fluorescein isothiocyanate (FITC)-conjugated Anti-PPV antiserum.

EXPERIMENTAL DESIGN:

All of the procedures involved in performance of this study are described in a detailed series of SOPs that are maintained at MicroBioTest, Inc.

A. Inoculum preparation:

Viral stocks are secured from reputable sources and are propagated at MicroBioTest, Inc. Records are maintained that demonstrate the origin of the virus. The viral stocks are titered, and stored at or below –70C.

Frozen viral stocks will be thawed on the day of the test (fresh stock cultures may be used also).

B. Test sample preparation and testing:

The volume of blood components, size of bags, and doses delivered in this experimental plan have been selected by the sponsor of the study to provide maximum overlap between earlier and later experiments as directed by T[3]I. To minimize handling of contaminated samples, the plan requires that each transfer bag be sampled once only. Controls, filled and sampled but not irradiated, are used to establish a non-treated baseline. Both plasma and platelets will be used.

1. Fresh Frozen Plasma (FFP):

Two units of FFP, volume ~ 300 ml each, will be divided into eleven 50 mL samples in 150 mL T[3]I supplied transfer bags and spiked with 10% of the challenge virus (see below).

Sample No.	FFP in 150 mL bags (mL)	Description of Irradiation J/cm^2
1	50	A "process control"
2	50	B
3	50	C
4	50	D
5	50	E
6	50	F
7	50	G
8	50	H
9	50	I
10	50	J
11	~50	K "not spiked" cytotoxicity and viral interference

Note: "B-K" will be processed by the sponsor (T3I) and MicroBioTest has no knowledge of the level of the treatment process. "A" receives no treatment.

Post-treatment, the irradiated samples will be assayed for the presence of any residual infectious virus.

2. Platelets:

Three units of random platelet concentrates, volume ~ 65 ml each, will be divided into nine 20-mL samples in 150 ml T^{3}I supplied transfer bags and spiked with 10% of the challenge virus (see below).

Sample No.	Platelets in 150 mL bags (mL)	Description of Irradiation J/cm^2
1	20	L "process control"
2	20	M
3	20	N
4	20	O
5	20	P
6	20	Q
7	20	R
8	20	S
9	~20	T "not spiked" cytotoxicity and viral interference

Note: "M-T" will be processed by the sponsor (T3I) and MicroBioTest has no knowledge of the level of the treatment process. "L" receives no treatment.

Post-treatment, the irradiated samples will be assayed for the presence of any residual infectious virus.

C. Test apparatus:

The T^{3}I apparatus will be delivered and installed at MicroBioTest (MBT) by T^{3}I technicians. The virology laboratory will prepare the spiked test bags of both FFP and platelets and hand over to T^{3}I technicians (prior to delivery of bags for irradiation, biosafety issues will be discussed with T^{3}I team). The T^{3}I team will irradiate the bags and return them to virology laboratory for infectious PPV determination. The sponsor will hold all of the irradiation-related information.

D. Analysis of samples:

Following irradiation of both sets of FFP and platelet samples, the T^{3}I team will deliver the samples to virology laboratory.

All the spiked samples will be serially diluted tenfold in cell culture medium (CCM) following SOP 1007.2 (1) and plated on semi-confluent monolayers of host cells (see below). Each dilution will be plated quadruplicate.

E. Cell culture:

Selected dilutions of the samples will be added to semi-confluent monolayers of host cells and incubated at 37±2C with 5±1% CO_2 for 60 to 90 minutes for viral adsorption. Post-adsorption, the monolayers will be washed once with Earle's balanced solution (EBSS) and incubated for an additional period of 4-7 days under the conditions given above. Four determinations will be recorded for each dilution of both tests and controls.

Post-incubation the infectious PPV will be assayed by immunofluorescence assay (IFA) (see below).

F. Immunofluorescence assay:

Post-incubation phase, the cell media will be aspirated and monolayers washed with PBS. The plates will be fixed with TC grade alcohol and left in a cold room until stained with FITC-conjugated Anti-PPV antiserum. The data thus obtained will be used to determine the fluorescent focus forming unit dose 50% per mL ($FFFU_{50}$/mL) using the Reed and Muench[1] method. The results of the tests will be compared with un-treated PPV-spiked FFP and platelet controls (see below).

F. Controls:

1. Viral interference in irradiated or non-irradiated FFP and platelet control (K and T "not spiked"):

   This control will determine if irradiated or non-irradiated FFP and platelets interfere with PPV replication *in vitro*. To achieve this, equal volumes of either irradiated or non-irradiated FFP and platelet samples will be mixed with PPV and incubated for one to two hours, serially diluted and plated in host cell containing plates. This control will compared with a virus sample similarly treated except the blood products replaced with CCM.

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[1]. Reed, L.J and Muench, H.A 1938. A simple method of estimating fifty percent end points. Am. J. of Hyg., 27: 493-497.

Post-incubation these plates will be processed for determination of infectious PPV (see above). The non-irradiated FFP and platelet controls will be performed prior to initiating the test.

2. Cytotoxicity (K and T "not spiked"):

The cytotoxic effect of irradiated or non-irradiated FFP and platelet control will be determined in two parts. First, both irradiated or non-irradiated FFP and platelet controls will be serially diluted, plated on host cells and their cytotoxicity will be determined microscopically. Second, serially diluted samples of both irradiated or non-irradiated FFP and platelet control will be plated on the host cell plate and incubated for one to two hours at 37±2C with 5±1% CO_2. Post-incubation, the plates will be aspirated and washed once with EBSS and serially diluted PPV will be plated and incubated with the rest of the controls.

This control will be compared with a virus sample similarly treated except the blood products will be replaced with CCM. This control will determine if either irradiated or non-irradiated FFP and platelet alter the permissibility of the host cells to initiate PPV infection. The non-irradiated FFP and platelet controls will be performed prior to initiating the test.

3. Process controls (A and L):

To compare viral inactivation during the various process treatments of both FFP and platelet samples a non-treated bag of both FFP and platelets will be spiked with PPV. Samples from these bags will be serially diluted, plated, incubated and post-incubation processed with test samples. The results from these controls will be compared with test results to determine the $\log_{10}$ viral inactivation in irradiated samples. These controls will be performed with test samples. These controls will determine the infectivity of virus post-spiking in non-irradiated FFP and platelets.

The titer of infectious PPV recovered will be compared directly with virus recovered from the processed sample test samples of both FFP and platelet. These controls will be performed at the same time as the test.

4. Virus stock titer:

In order to confirm the original virus titer, an aliquot of each virus inoculum will be serially diluted, plated on the host cell plates, incubated and post-incubation processed with the test plates. This control will be performed prior to initiation of the test.

5. Cell viability control:

Four wells will be inoculated with CCM only and processed in the same manner as the test plate during the incubation and post-incubation phase of the study. This control will demonstrate that cells remain viable throughout the course of the assay period. In addition, it will confirm the sterility of the cell culture media employed throughout the assay period.

G. Calculation:

The FFFUD_{50}/mL will be determined using the method of Reed and Muench, Am. J. of Hyg. 1938, 27:493. The test results shall be reported as PPV inactivation due to the process treatment and expressed as log_{10} inactivation of infectious PPV.

TEST EVALUATION CRITERIA

According to the sponsor of the study, the compound passes the test if there is a 4-5 log_{10} reduction when test samples are compared to the Process controls of both FFP and platelets respectively.

TEST ACCEPTANCE CRITERIA:

The test will be acceptable for evaluation of the test results if the criteria listed below are satisfied. The study director may consider other causes that may affect test reliability and acceptance.

- Virus must be recovered in the 10^{-6} dilution of the Process controls.
- Viral-induced cytopathic effects must be distinguishable from the cytotoxic effects of both irradiated and non-irradiated FFP and platelet controls.

DATA PRESENTATION:

The final report will include the following information (if appropriate) for both the test and control cultures:

- Virus stock titer
- Test results
- Controls: process, cytotoxicity and viral interference
- Assay system.

STUDY DATES:

The anticipated date of study initiation (date when the study director signs the protocol) is within three weeks from receipt of letter of authorization with a purchase order number and signed protocol. The final report will be submitted to the sponsor upon study completion.

PERSONNEL AND TESTING FACILITIES:

A study director will be assigned prior to initiation of the test. Resumes are maintained and are available on request. This study will be conducted at MicroBioTest, Inc., 105B Carpenter Drive, Sterling, Virginia 20164.

RECORDS TO BE MAINTAINED:

All raw data, protocol, protocol modifications, test material records, final report, and correspondence between MicroBioTest and the sponsor will be stored in the archives at MicroBioTest, Inc., 105B Carpenter Drive, Sterling, Virginia 20164

All changes or revisions to this approved protocol will be documented, signed by the study director, dated and maintained with this protocol. The sponsor will be notified of any change, resolution, and impact on the study as soon as practical.

The proposed experimental start and termination dates; additional information about the test agent; challenge virus and host cell line monolayer used; and the type of neutralizer to be employed in the test will be addressed in a project sheet issued separately for each study.

TEST SUMMARY

TITLE: Evaluation of T^3I Pathogen Inactivation System – Using *Porcine parvovirus*

STUDY DESIGN: This study was performed according to the signed protocol and project sheets issued by the study director.

See Project Sheets (Appendix I)
See signed protocol (Appendix II)

TEST MATERIALS: T3I "Hemalight" system, received at MicroBioTest, Inc. REDACTED and assigned DS No. 5344

SPONSOR: Triton Thalassic Technologies, Inc.
241 Ethan Allen Highway
Ridgefield, CT 06877

TEST CONDITIONS

Challenge virus:

Porcine parvovirus, American BioResearch Laboratories, Seymour, TN / VMRD, Inc., Pullman, WA

Host:

PT-1 cells, American BioResearch Laboratories, Seymour, TN / VMRD, Inc., Pullman, WA

Active ingredient in test product:

Light

Neutralizer used:

N/A

Contact time:

Not known

Contact temperature:

Ambient

Media and reagents:

Earle's Balanced Salt Solution
Eagle Minimum Essential Medium containing 10% fetal bovine serum (CCM)
Fresh frozen plasma (FFP)
Platelets
Phosphate Buffered Saline
Tissue culture grade alcohol
FITC-conjugated Anti-PPV antiserum

STUDY DATES AND FACILITIES

The laboratory phase of this test was performed at MicroBioTest, Inc. 105B Carpenter Drive, Sterling, VA 20164, on two separate dates. The pre-test controls, consisting of viral interference and cytotoxicity controls (using non-irradiated FFP and platelets), input virus control and virus stock titer were tested from REDACTED The actual test with controls was tested from REDACTED The study director signed the protocol REDACTED The study completion date is the date the study director signed the final report.

All changes or revisions of the protocol were documented, signed by the study director, dated and maintained with the protocol.

RECORDS TO BE MAINTAINED

All testing data, protocol, protocol modifications, test material records, the final report, and correspondence between MicroBioTest, Inc. and the sponsor will be stored in the archives at MicroBioTest, Inc., 105B Carpenter Drive, Sterling, VA 20164.

RESULTS

Results and data are presented in the Tables 1 – 10. The process controls met the established criteria for a valid test. The 50% fluorescent foci forming unit dose / mL was determined using the method of Reed and Muench, 1938.

RESULTS (continued)

Pre-Test Controls

Table 1
Sample "K" non-irradiated FFP

Dilutions	Viral Interference	Cytotoxicity Part 1	Cytotoxicity Part 2
10^{-1}	+ + + +	0 0 0 0	+ + + +
10^{-2}	+ + + +	0 0 0 0	+ + + +
10^{-3}	+ + + +	0 0 0 0	+ + + +
10^{-4}	+ + + +	ND	+ + + +
10^{-5}	+ + + +	ND	+ + + +
10^{-6}	+ + + +	ND	+ – – +
10^{-7}	– – – –	ND	– – – –
10^{-8}	– – – –	ND	– – – –
10^{-9}	– – – –	ND	– – – –
10^{-10}	– – – –	ND	– – – –
$FFFUD_{50}$/mL	$10^{6.50}$	NA	$10^{6.00}$

Table 2
Sample "T" non-irradiated Platelets

Dilutions	Viral Interference	Cytotoxicity Part 1	Cytotoxicity Part 2
10^{-1}	+ + + +	0 0 0 0	+ + + +
10^{-2}	+ + + +	0 0 0 0	+ + + +
10^{-3}	+ + + +	0 0 0 0	+ + + +
10^{-4}	+ + + +	ND	+ + + +
10^{-5}	+ + + +	ND	+ + + +
10^{-6}	+ + + +	ND	+ + + +
10^{-7}	+ + + +	ND	– – – –
10^{-8}	– – – –	ND	– – – –
10^{-9}	– – – –	ND	– – – –
10^{-10}	– – – –	ND	– – – –
$FFFUD_{50}$/mL	$10^{7.50}$	NA	$10^{6.50}$

Key: + = PPV infected cells were detected
– = PPV infected cells were not detected
0 = indicates no cytotoxicity was observed
ND = not determined
NA = not applicable

RESULTS (continued)

Pre-Test Controls

Table 3

Input Virus / Virus Stock Titer

Dilutions	Input Virus control	Virus stock titer (VST)
10^{-1}	+ + + +	+ + + +
10^{-2}	+ + + +	+ + + +
10^{-3}	+ + + +	+ + + +
10^{-4}	+ + + +	+ + + +
10^{-5}	+ + + +	+ + + +
10^{-6}	+ + + +	+ + + +
10^{-7}	+ − + +	+ + + +
10^{-8}	− − + −	+ + − +
10^{-9}	− − − −	− − − +
10^{-10}	− − − −	− − − −
$FFFUD_{50}$/mL	$10^{7.50}$	$10^{8.50}$

Table 4

Cell Viability Control
− − − −

Key: + = PPV infected cells were detected
− = PPV infected cells were not detected
0 = indicates no cytotoxicity was observed
ND = not determined
NA = not applicable

RESULTS (continued)

Test Results

Table 5

Dilutions	FFP Samples								
	B	C	D	E	F	G	H	I	J
10^{-1}	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -
10^{-2}	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -
10^{-3}	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -
10^{-4}	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -
10^{-5}	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -
10^{-6}	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -
10^{-7}	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -
10^{-8}	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -
10^{-9}	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -
10^{-10}	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -
$FFFUD_{50}$/ mL	0	0	0	0	0	0	0	0	0

Table 6

Dilutions	Platelet Samples						
	M	N	O	P	Q	R	S
10^{-1}	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -
10^{-2}	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -
10^{-3}	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -
10^{-4}	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -
10^{-5}	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -
10^{-6}	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -
10^{-7}	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -
10^{-8}	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -
10^{-9}	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -
10^{-10}	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -
$FFFUD_{50}$/ mL	0	0	0	0	0	0	0

Key: + = PPV infected cells were detected
– = PPV infected cells were not detected
0 = indicates no cytotoxicity was observed
ND = not determined
NA = not applicable

RESULTS (continued)

Controls

Table 7

Sample "K" irradiated FFP

Dilutions	Viral Interference	Cytotoxicity Part 1	Cytotoxicity Part 2
10^{-1}	+ + + +	0 0 0 0	+ + + +
10^{-2}	+ + + +	0 0 0 0	+ + + +
10^{-3}	+ + + +	0 0 0 0	+ + + +
10^{-4}	+ + + +	0 0 0 0	+ + + +
10^{-5}	+ + + +	0 0 0 0	+ + + +
10^{-6}	− + + +	0 0 0 0	+ + − +
10^{-7}	− − − −	ND	− − − −
10^{-8}	− − − −	ND	− − − −
10^{-9}	− − − −	ND	− − − −
10^{-10}	− − − −	ND	− − − −
$FFFUD_{50}$/mL	$10^{6.33}$	NA	$10^{6.33}$

Table 8

Sample "T" irradiated Platelets

Dilutions	Viral Interference	Cytotoxicity Part 1	Cytotoxicity Part 2
10^{-1}	+ + + +	0 0 0 0	+ + + +
10^{-2}	+ + + +	0 0 0 0	+ + + +
10^{-3}	+ + + +	0 0 0 0	+ + + +
10^{-4}	+ + + +	0 0 0 0	+ + + +
10^{-5}	+ + + +	0 0 0 0	+ + + +
10^{-6}	+ − − +	0 0 0 0	+ − − −
10^{-7}	− − − −	ND	− − − −
10^{-8}	− − − −	ND	− − − −
10^{-9}	− − − −	ND	− − − −
10^{-10}	− − − −	ND	− − − −
$FFFUD_{50}$/mL	$10^{6.00}$	NA	$10^{5.67}$

Key: + = PPV infected cells were detected
− = PPV infected cells were not detected
0 = indicates no cytotoxicity was observed
ND = not determined
NA = not applicable

RESULTS (continued)

Table 9
Process controls / Input virus / Viral stock titer

Dilutions	Sample A	Sample L	Input virus	Viral Stock Titer
10^{-1}	++++	++++	++++	++++
10^{-2}	++++	++++	++++	++++
10^{-3}	++++	++++	++++	++++
10^{-4}	++++	++++	++++	++++
10^{-5}	+−++	−−++	++++	++++
10^{-6}	+−−+	−−−−	++++	++++
10^{-7}	−−−−	−−−−	−−++	++++
10^{-8}	−−−−	−−−−	−−−−	+−+−
10^{-9}	−−−−	−−−−	−−−−	−−−−
10^{-10}	−−−−	−−−−	−−−−	−−−−
$FFFUD_{50}$/mL	$10^{5.77}$	$10^{5.00}$	$10^{7.00}$	$10^{8.00}$

Table 10

Cell Viability Control
−−−−

Key: + = PPV infected cells were detected
− = PPV infected cells were not detected
0 = indicates no cytotoxicity was observed
ND = not determined
NA = not applicable

CONCLUSION

When *Porcine parvovirus* (PPV)-spiked fresh frozen plasma (FFP) and platelet bags were exposed to T3I "Hemalight" system by the sponsor, T3I "Hemalight" system inactivated ≥ 5 $\log_{10}$ of infectious PPV, as compared to the process controls. The process controls met the Test Acceptance Criteria stated in the protocol for a valid test. These conclusions are based on observed data.